

# The degree of plant resilience to infection correlates with virus virulence and host-range

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## Abstract

Antagonistic interactions between plant viruses and their host lead to coevolution of virus virulence and host defenses. Plant virus adaptation to the defenses of a specific host may occur in detriment of their ability to exploit alternative hosts, driving to specialization. Using comparative analyses of the symptoms induced by members of several families of RNA plant viruses and of a quantitative estimate of the susceptibility to viral infection of plant families, it is shown that viral families that infect hosts from different families (generalists) exploited them in a benign way. Furthermore, plants infected by generalist viruses showed, on average, a large susceptibility to infection. By contrast, viral families parasitizing a small number of hosts from very few families (specialists) exploited them more virulently. Plants hosting very virulent and specialized viruses are also less susceptible to viral infection. Finally, it has been shown that specialist viruses are, on average, more virulent than generalists ones.

**Additional key words:** comparative method, evolution of virulence, host-range, plant virus, virus ecology and evolution.

## Resumen

### El grado de resistencia a la infección de las plantas correlaciona con la virulencia y gama de hospedadores de los virus

Las interacciones antagonísticas que se establecen entre los virus de plantas y sus hospedadores conducen a una coevolución entre la agresividad viral y las defensas de las plantas. La adaptación de un virus a las defensas de un hospedador particular puede resultar en una disminución de su capacidad para infectar eficientemente a otros hospedadores alternativos, conduciendo a un mayor grado de especialización. Empleando un análisis comparado de los síntomas inducidos por los miembros de varias familias de virus de plantas y de una estima cuantitativa de la susceptibilidad a la infección de distintas familias de plantas, se ha observado que aquellos virus con una amplia gama de familias hospedadoras (generalistas) son, en promedio, menos virulentos. Más aún, estas familias de plantas muestran, en promedio, una mayor susceptibilidad a la infección. Por el contrario, aquellos virus que parasitan un reducido número de hospedadores, pertenecientes a muy pocas familias (especialistas), las explotan de una manera más virulenta. Las familias de plantas infectadas por virus especialistas y virulentos también son menos susceptibles a la infección. Finalmente, se ha observado que, en promedio, los virus especialistas son más virulentos que los generalistas.

**Palabras clave adicionales:** ecología y evolución viral, evolución de la virulencia, gama de hospedadores, método comparativo, virus de plantas.

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## Introduction<sup>1</sup>

Hosts comprise the main environmental factor affecting the evolution of parasites (Ehrlich and Raven, 1964; Thompson, 1994; Combes, 2001). The degree to which parasites adapt to a particular host depends on the balance between within-host selection and among-host gene flow (Gandon *et al.*, 1996; Kaltz and Shykoff, 1998; Lajeunesse and Forbes, 2001; Dennehy *et al.*, 2006). Host specialization represents the reduction in the number of potential host species on which a parasite can successfully survive and reproduce. Such specialization implies that specialist parasites are better adapted to their main host than to alternative ones. The advantages of generalism are not well understood; it has been suggested that evolution should favor specialists because there are trade-offs that limit the fitness of generalists in any of the alternative hosts or because evolution proceeds faster with narrower niches (Whitlock, 1996; Woolhouse *et al.*, 2001). It is widely accepted that adaptation to a specific environment is often coupled with fitness losses in alternative environments simply because mutations that are beneficial in the first might be deleterious (Kawecki, 1994; Kassen, 2002), neutral, or even under weak positive selection (Fry, 1996) in the alternative. This antagonistic pleiotropy could limit the range of adaptation and promotes the evolution of specialization (Futuyma and Moreno, 1988; Duffy *et al.*, 2006). In this regard, host heterogeneity has been suggested as a main force maintaining parasites diversity (Bell, 1997; Kassen and Bell, 1998; MacLean, 2005). Contrastingly, host homogeneity promotes the evolution of specialist genotypes, reduces genetic variability for fitness, and limits gene flow among parasite populations, thereby enhancing local adaptation and speciation (Futuyma and Moreno, 1988; Thompson, 1994; Woolhouse *et al.*, 2001).

Plants are not passive victims of their parasites, but coevolve with them to provide efficient defenses (e.g., gene silencing, systemic acquired resistance, or hypersensitive local responses) that limit, reduce or eliminate the damage caused by parasites (Ehrlich and Raven, 1964; Ratcliff *et al.*, 1997; Woolhouse *et al.*, 2001, 2002). The role of the host and its anti-parasite defenses in parasite specialization remains largely undetermined.

All in all, plants and their parasites shall be involved in an arms-race coevolutionary dynamic in which both players should evolve to keep pace each other. Rather than being an exception, plant viruses commonly infect multiple host species, in many cases host species belong to different, even unrelated, plant families. Although it has been recently shown that generalist plant viruses do not distribute randomly across all their potential hosts but tend to associate preferentially with a particular one (Malpica *et al.*, 2006). Concurrently, it has been recently shown that adaptation of PPV to an herbaceous host comes with an infectivity cost in the original one (Wallis *et al.*, 2007). Since the machinery required for infection, exploitation, and transmission likely varies among plant hosts, the selective pressures acting over a plant virus on different hosts may vary as well. When confronted with a multiple host situation, selection may favor two different exploitation strategies of each available host. First, viruses may become more virulent (i.e., evolving a more intense exploitation of the host plant) for the better-quality host. Alternatively, viruses may evolve host-choice strategies to predominantly infect the best possible host (i.e., evolving a less intense exploitation but a more efficient transmission). Several epidemiological studies have shown how the multiplicity of potential hosts can affect the dynamics of infectious diseases (Woolhouse *et al.*, 2001, 2002). This sort of knowledge may be of help to design intervention measures to control disease propagation and identifying and managing the reservoirs of these generalist parasites.

Here the results from a comparative study in which the virulence of plant viruses across different host plant families that they infect are reported. Viral species included in the study belong to four families having a positive-sense and single-stranded RNA genome (*Bromoviridae*, *Comoviridae*, *Potyviridae*, and *Tombusviridae*) plus to the family *Caulimoviridae* of pararetroviruses for which RNA also plays a central role during their infectious cycle. The election of these five families was purely random, although the sample is quite representative since it contains 29% of all plant virus families, which in turns represents up to 46% of plant virus genera. In the following, we will use the word virulence as the relative amount of damage caused to

<sup>1</sup> Abbreviations used: BiMoV (*Bidens mottle virus*), *I* (virus incidence in a plant family), df (degrees of freedom), ICTV (International Committee on Taxonomy of Viruses), PDV (*Prune dwarf virus*), PPV (*Plum pox virus*), RYMV (*Rice yellow mottle virus*), *S* (susceptibility of a plant family), SEM (standard error of the mean), TEV (*Tobacco etch virus*), TSV (*Tobacco streak virus*), TYLCV (*Tomato yellow leaf curl virus*),  $\Lambda$  (likelihood-ratio test statistic).

a host by a given virus (Shaner *et al.*, 1992). A virulence index based upon the severity of symptoms induced by the virus has been developed and used to scale the virulence of each virus into each potential host. The present study will focus in addressing the following questions: (i) Defining as specialists those viruses found infecting a single plant family and as generalists those able to infect hosts from different families, does specialization come along with an increase in virulence? (ii) After defining a susceptibility index for each host plant family, the association between host susceptibility and virus virulence was explored. (iii) Using the plant susceptibility index and the frequency of specialist viruses, the correlation between plant susceptibility and virus specialization has been studied.

## Material and Methods

### Data mining

Information on the number of susceptible species for members of each viral genus belonging to the *Bromoviridae*, *Caulimoviridae*, *Comoviridae*, *Potyviridae*, and *Tombusviridae* families of plant viruses was obtained from the following internet resources: Plant Virus Online Database (<http://micronet.im.ac.cn/vide>), the Universal Virus Database of the ICTV (<http://www.ncbi.nlm.nih.gov/ictvdb/index.htm>), and the Virus Taxonomy Online (<http://www.virustaxonomyonline.com>). For each virus species, this database provides information on host range and symptoms. Plant families are also classified according to whether they contain susceptible hosts.

### The virulence index

Different viruses produce different symptoms on different plants. For the comparative study undertaken, it would be desirable to translate symptoms into a common quantitative scale that reflect the impact of viral infection on the host plants. Table 1 shows the symptoms scale used to assign a virulence value to each virus species on each host. Values were additive according to the presence of multiple symptom. The scale was inspired on previous symptoms scales proposed for viruses such as TYLCV (Delatte *et al.*, 2006) or RYMV (Sorho *et al.*, 2005). The following two examples illustrate how virulence was computed. First, TSV

**Table 1.** Weight of symptoms according to their severity

Symptomless infection +0	
Local symptoms +1	
Chlorotic +0.1	Spots +0.01
Necrotic +0.2	Ringspots +0.02
Systemic symptoms +2	
Mottling, etching and mosaic +0.1	
Chlorosis, and yellowing +0.2	
Streaking and blistering +0.3	
Necrosis +0.4	
Stunting, premature senescence, leaf malformation and other developmental abnormalities +0.5	
Wilting +0.6	
Death +3	

*Ilarvirus* has been described naturally infecting several different hosts. For instance, on *Nicotiana tabacum*, TSV produces systemic necrosis, and hence we assigned a virulence of 2 (systemic) +0.4 (necrosis) = 2.4. On *Vigna unguiculata*, TSV produces a local infection (+1) with symptoms ranging from chlorotic spots (+0.1 + 0.01) to necrotic spots (+0.2 + 0.01) and, therefore, its virulence on this host was quantified as the median value of 1.11 and 1.21, that is 1.16. Using these figures, the virulence of a given virus across host plants can be quantitatively compared: TSV is 2.1-times more virulent in *N. tabacum* than in *V. unguiculata*. The second example, TEV *Potyvirus* produces systemic (+2) leaf mottling (+0.1) and necrotic etching (+0.4) in *N. tabacum* and, thus, its virulence on this host is 2.5. In *Datura stramonium*, another naturally infected host, TEV produces a systemic infection (+2) with leaf mottling (+0.1), vein banding (+0.2) and malformations in leafs and fruits (+0.5) and thus its virulence on this host can be quantified as 2.8. These figures can be used to compare the effect that different viruses produce in the same plant: TEV infection is 4.2% more virulent than TSV infection on tobacco plants. This virulence index ranges between 0.0 (avirulent) and 3.0 (deathly infection). When data existed for several hosts belonging to the same plant family, virulence was expressed as the median value across hosts. Obviously, this scale is somehow arbitrary and its biological meaning can be criticized. Nonetheless, it reflects the objective fact that viruses producing local lesions impact plants viability in a lesser extent than viruses propagating systemically; and among the later, those producing a general chlorosis would be less prejudicial than those inducing wilting.

The five virus families were separately analyzed. For each family, the number of plant families containing susceptible hosts and the median virulence of the member viruses on each host family were recorded. Then the effect that the degree of virus specialization (i.e., the number of hosts in which a virus successfully replicate) has on its virulence was evaluated. To do so, two different statistical tests were computed. First, a partial correlation coefficient, using viral genus as control variable, was computed between virulence and number of susceptible host plant families. Second, a two-sample *t* test was used to compare the virulence of generalist (> 1 host plant family) versus specialist (only one host plant family) classes of viruses. This two significance tests are testing the same scientific (but not statistical) hypothesis, i.e., are specialists more virulent? Each test produces a probability value for the particular outcome, assuming the null hypothesis to be correct. Combining these two probabilities, an overall test for significance can be computed by using Fisher's method (Sokal and Rohlf, 1995).

Obviously, this comparative approach has to take into consideration the phylogenetic relationships between viral genera within families. In other words, the potential problem of the non-statistical independence of the data should be addressed prior to running the above analyses. We applied Felsenstein's phylogenetically independent contrasts to test whether correlations between traits were simply due to shared ancestry or to a real coevolutionary process (Felsenstein, 1985). The method was used as implemented in the CONTRAST program of the PHYLIP package version 3.6 (available at <http://evolution.gs.washington.edu/phylip.html>). CONTRAST computes a likelihood-ratio test ( $\Lambda$ ) to assess whether incorporating phylogenetic information improves the explanation of the phenotypic variation. A non-significant  $\Lambda$  would imply that data can be considered as phylogenetically independent. The phylogenetic relationships within each family were those proposed by the ICTV in its 7<sup>th</sup> report and are available throughout the internet in (<http://www.virustaxonomyonline.com>).

### The host family susceptibility index

A total of 73 different plant families were infected by the viruses here considered. To numerically assess whether a given plant family was more prone to viral infection than others, we proceeded as follows. For each plant family, we computed the fraction out of the 202 viruses considered in the study that infected at

least one member of the family,  $I_j$  ( $j = 1, 2, \dots, 73$ ). For example, this fraction for the family *Acanthaceae* is  $2/202 = 0.0099$ , since only PDV and BiMoV infect at least one member of the *Acanthaceae*. Then, the susceptibility of the  $j^{\text{th}}$  plant family was defined as the probability of being infected by a virus in the current dataset:

$$S_j = I_j / \sum_{i=1}^{73} I_i. \quad \text{This index ranges from 0.0 (non-}$$

susceptible) to 1.0 (susceptible to all possible viruses).

This index can be affected by two biasing sources. First, obviously, by the number of plant species of agronomic interest belonging to each family: families containing members of agronomical interest (e.g., *Cucurbitaceae* or *Solanaceae*) will be better studied than plant families with few or no members of economical interest. Second, by the number of genera on each plant family: the more genera, the more likely for a virus to infect, simply by chance, at least one member of the family. However, this second source of bias can be discarded since a correlation test shows that the number of genera within a family do not affect the index ( $r = 0.1377$ , 71 df,  $P = 0.2557$ ).

All statistical tests were done using SPSS version 12.0.1 program (SPSS Inc.). Excel files with all the data are available upon request.

## Results

### Virulence and degree of virus specialization

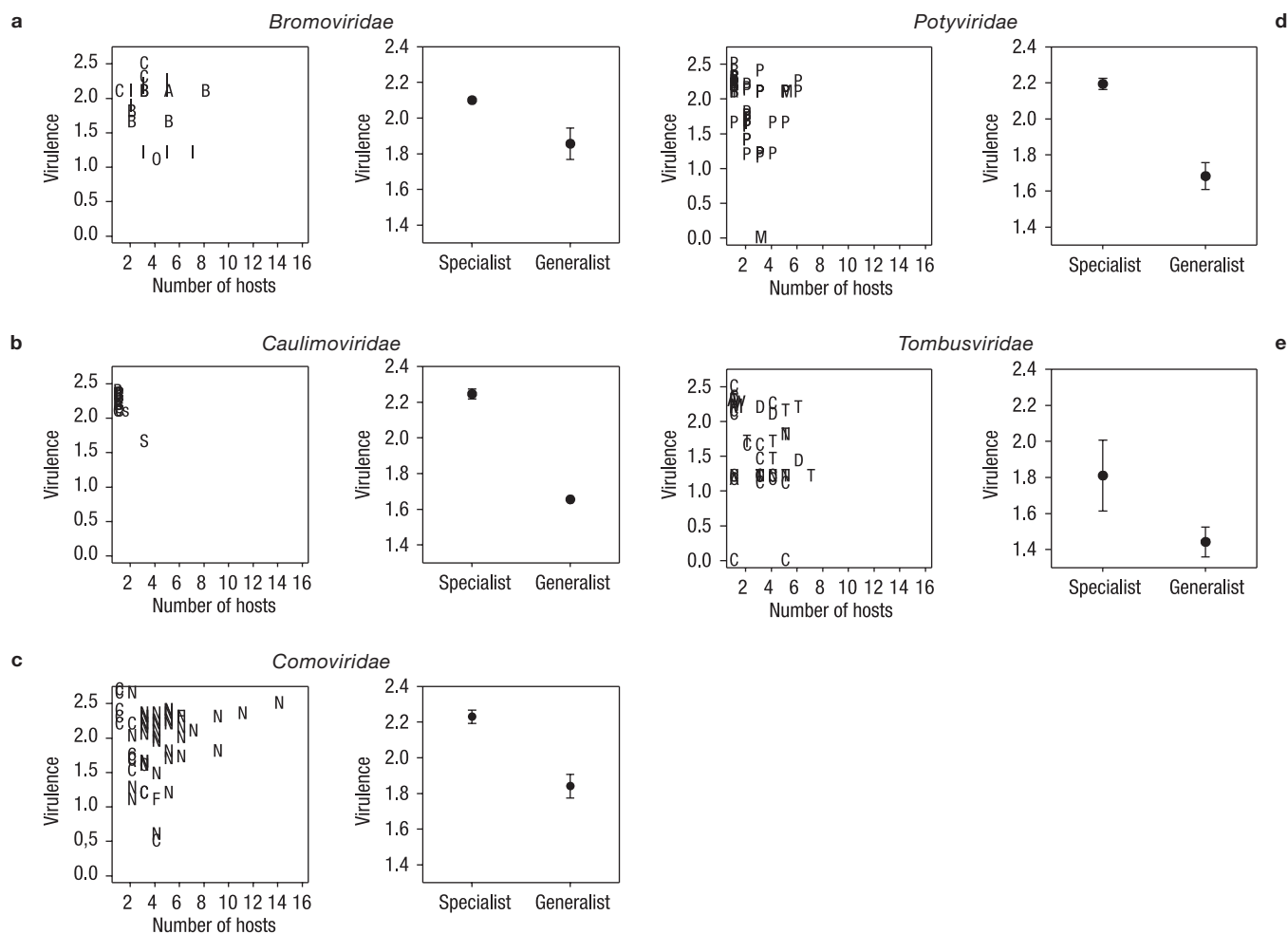
The first hypothesis tested was whether specialist viruses are, on average, more virulent than generalist ones. Gene-for-gene coevolution models (Frank, 1992, 1993a,b; Gandon and Michalakis, 2000; Sasaki, 2000) predict that viruses should become more and more virulent as they coevolve with plants simply to overcome their adaptive defenses. Therefore, a negative correlation is expected between virulence and the number of hosts susceptible to the infection of a given virus. Consequently, all significance tests will be reported as 1-tailed. Table 2 shows the results of all the statistical tests discussed hereafter. Data from the different genera within all viral families can be considered as independent according to the likelihood ratio test (in all cases,  $P \geq 0.0730$ ) and, therefore, the correlation analyses were performed.

The expected negative correlation has been obtained in three cases (Table 2): *Caulimoviridae* (Fig. 1B), *Potyviridae* (Fig. 1D), and *Tombusviridae* (Fig. 1E).

**Table 2.** Results of the statistical analyses

	Likelihood ratio test ( $\Delta$ ) of phylogenetic independence among genera			Partial correlations between virulence and number of hosts			Differences in virulence between specialists and generalists		
	$\Delta$	df	$P$	$r$	df	1-tailed $P$	$t$	df	1-tailed $P$
<i>Bromoviridae</i>	0.9028	3	0.8248	-0.2096	23	0.1574	2.7595*	23	0.0056
<i>Caulimoviridae</i>	0.2917	3	0.9616	-0.8142	11	0.0004	5.7615	12	<0.0001
<i>Comoviridae</i>	0.9444	3	0.8147	0.1156	52	0.9967	5.1026*	38.0549	<0.0001
<i>Potyviridae</i>	0.9665	3	0.0730	-0.2570	59	0.0228	6.3829*	50.3829	<0.0001
<i>Tombusviridae</i>	3.0591	3	0.3826	-0.3026	42	0.0230	2.0540	43	0.0231

\* Variances among groups were heterogeneous (Levene's test  $P < 0.05$ ) and the Welch's approximate  $t$ -test has been employed.



**Figure 1.** Relationship between virulence and host range (number of plant families whose members are susceptible to infection) for viruses belonging to the five families under study. The left column contains the raw data for each genus within each family. The right column shows the differences in virulence after classifying viruses as specialists or generalists. (a) *Bromoviridae* genera are indicated as: I, *Ilarvirus*; B, *Bromovirus*; C, *Cucumovirus*; A, *Alfavirus*; and O, *Oleavirus*. (b) *Caulimoviridae* genera are indicated as: S, *Soymovirus*; C, *Caulimovirus*; Cs, *Cavemovirus*; P, *Petuvirus*; R, *Tungrovirus*; and S, *Soymovirus*. (c) *Comoviridae* are indicated as: C, *Comovirus*; F, *Fabavirus*; and N, *Nepovirus*. (d) *Potyviridae* are labeled as: B, *Bymovirus*; I, *Ipomovirus*; M, *Macluravirus*; P, *Potyvirus*; R, *Rymovirus*; and T, *Tritimovirus*. Finally, (e) the *Tombusviridae* genera are labeled as: A, *Aureusvirus*; Av, *Avenavirus*; C, *Carmovirus*; D, *Dianthovirus*; M, *Machlovirus*; N, *Necrovirus*; P, *Panicovirus*; and T, *Tombusvirus*. Error bars represent the standard error of the mean (SEM).



However, after classifying all virus species as specialist or generalists, the two-sample *t* tests were significant for all five viral families (Table 2). Regarding the magnitude of the difference, for the *Bromoviridae*, generalists were, on average, 11.6% less virulent (Fig. 1A); generalist caulimovirus were 31.0% less virulent; *Comoviridae* generalists were 17.4% less virulent (Fig. 1C); generalist potyviruses were 23.5% less virulent; and, finally, the virulence of the generalist tombusviruses was 20.4% lower than for the specialist members of this family.

The two viral families in which the two tests disagreed need to be explored more carefully. In the case of the family *Bromoviridae*, after applying Fisher's method for combining probabilities from independent tests of significance, overall, specialist bromoviruses were more virulent than generalists ones ( $P = 0.0071$ ). In the case of the family *Comoviridae*, it is worth noting that this lack of correlation is entirely due to the existence of four nepoviruses having hosts in more than eight plant families but still showing high virulence (left panel Fig. 1C). If these cases are removed from the dataset, then a significant negative partial correlation is obtained ( $r = -0.6758$ , 48 df, 1-tailed  $P < 0.0001$ ). Furthermore, Fisher's method confirmed that, overall, specialist comoviruses were more virulent than specialist ones ( $P < 0.0001$ ).

All five viral families analyzed showed the predicted negative relationship in at least one of the two statistical tests employed, and always after combining the result of both tests. Furthermore, when the partial correlation coefficient computed for each family was treated as a single observation, the average correlation coefficient,  $-0.2592$ , was significantly negative (Jackknife resampling test:  $P = 0.0017$ ). In good agreement, when the relative differences between specialists and generalists estimated for each family were treated as single observations, the median difference was significantly greater than zero (0.2011; Jackknife resampling test:  $P = 0.0009$ ). In conclusion, when data from viruses belonging to different viral families are combined, a general trend arises suggesting a negative correlation between virulence and host-range, as predicted by the gene-for-gene coevolution hypothesis.

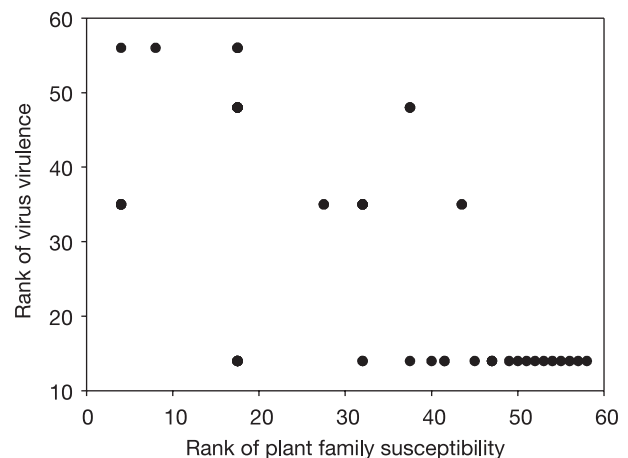
### Plant susceptibility to infection and viral virulence

Next, it was explored whether the degree of virulence characterizing a given virus was related with the susceptibility of the plants it naturally infects. According

to the gene-for-gene coevolution hypothesis, plants should evolve their defenses in response to virus counter-defenses (Frank, 1992, 1993a; Gandon and Michalakakis, 2000; Sasaki, 2000). Therefore, a negative correlation between the susceptibility of plants and the virulence of viruses is expected. In other words, the more resistant to infection is a plant (i.e., few viruses are able to infect and induce symptoms), the more virulent has to be a virus to overcome its defenses. To test this prediction, the following two steps procedure was applied. First, the susceptibility index for each plant family has been computed as described above. Second, the minimum virulence value among all viruses able to successfully infect at least one member of each plant family was obtained. This minimum virulence represents the lowest value associated with the virus' ability to overcome the plant defenses. Pairs of values were ranked and the resulting ranks were used to prepare the scatter plot shown in Figure 2. As predicted by the gene-for-gene coevolutionary hypothesis, a negative correlation exists between plant susceptibility and the minimum degree of virulence associated with a successful infection (Spearman's  $\rho_s = -0.5609$ , 71 df, 1-tailed  $P < 0.0001$ ). In other words, overall, more virulent viruses are associated with resistant plants whereas low virulent viruses are associated with permissive plants.

### Plant susceptibility to infection and the degree of viral specialization

Finally, it was tested whether the average degree of susceptibility of a plant family was associated with the



**Figure 2.** Relationship between the susceptibility to infection of different plant families and the minimum virulence necessary to overcome their defenses.

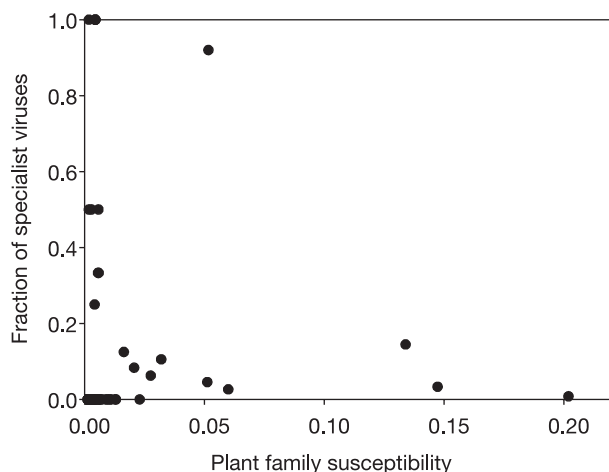
extent of specialization of the virus families infecting it. According to the gene-for-gene coevolutionary hypothesis, the more susceptible to infection a plant is the less specialization needs a virus to successfully infect and replicate. In other words, susceptible plants should be, on average, infected more frequently by generalist viruses whereas resistant plants should be mainly infected by specialized viruses. To look for the predicted negative correlation, it is first required to have an estimate of the average abundance of specialist viruses among those infecting each plant family. To do so, for each plant family the number of viruses infecting them that were specialist (i.e., were found only infecting plants from this family) and generalists (i.e., were found infecting plants from several families) was recorded. Pairs of values were used to prepare the scatter plot shown in Figure 3, which shows the fraction of specialist viruses as a function of plant family susceptibility. Out of 73 plant families studied, 50 were infected by generalist viruses whereas only 23 were infected by both specialist and generalist viruses. It is conceivable that specialist viruses have not been found in some, if not all, these 50 families simply because they have not been carefully screened for viruses. By contrast, the infectivity of specialist viruses on alternative hosts has been studied. Therefore, keeping in mind this possible source of bias, it was conservatively decided not to incorporate these 50 pairs into the correlation analysis (the data laying on abscises). As predicted by the gene-for-gene coevolution hypothesis, a significant negative correlation exists between plant susceptibility and the

abundance of specialist viruses infecting each plant family ( $\rho_s = -0.7519$ , 21 df, 1-tailed  $P = 0.0001$ ).

## Discussion

Does the radiation of plant viruses into multiple host plants affect their virulence? How does specialization into a single host plant affect virulence? Are susceptible plants associated with more/less virulent viruses? Here a comparative approach has been taken to address these questions. The advantage of such approach is that it allows inferring general trends without losing into the specific details and peculiarities of particular virus/plant associations. First, we found that viruses that adopted a specialist strategy and infect hosts from a single plant family are, on average, more virulent than viruses that had adopted a generalist strategy and are able to infect hosts from multiple plant families. This observation is in complete agreement with a recent report showing that virulence is higher in specialist malaria parasites than in generalist ones (Garamszegi, 2006). These observations are also in good agreement with the prediction of recent theoretical models seeking to explain the evolution of virulence of parasites facing multiple hosts (Ebert, 1998; Regoes *et al.*, 2000). According to these models, if virulence is proportional to the parasite's reproductive rate and it trade-offs across hosts (i.e., a parasite cannot replicate with maximum efficiency in all hosts and the better it does in one host, the worse it should perform in an alternative one), then host heterogeneity prevents virulence from increasing indefinitely; a situation that leads to intermediate levels of virulence across all host types. Alternatively, in the case of host homogeneity, within-host competition among pathogen strains is the main driving force and virulence rises up with no limit (reviewed by Ebert, 1998). In other words: generalist viruses should show intermediate levels of virulence across plant hosts whereas specialist ones should be very virulent in their host. However, evolution towards intermediate levels of virulence in heterogeneous hosts strongly depends upon the existence of trade-offs. If this assumption is removed from the models, then the outcome is completely different and virulence can increase with no limit even with host heterogeneity (Ganusov *et al.*, 2002). The results here presented support the validity of the trade-off assumption.

*In vitro* evolution experiments in which RNA viruses evolve in and adapt to animal cell cultures have provided good insights into the question of virus specialization.



**Figure 3.** Relationship between the susceptibility to infection of different plant families and the number of specialist viruses found to infect each plant family.

Several studies have shown that viral populations readily adapt to heterogeneous temporally fluctuating host cell types (Novella *et al.*, 1999; Weaver *et al.*, 1999; Turner and Elena, 2000). A common feature of all these studies is the fact that a viral population evolved under such conditions becomes a generalist without paying fitness costs in any of the alternating hosts. By contrast, viral populations evolved in a constant cell host become specialist, paying fitness costs in any alternative environment (Holland *et al.*, 1991; Novella *et al.*, 1999; Weaver *et al.*, 1999; Crill *et al.*, 2000; Turner and Elena, 2000; Cooper and Scott, 2001; Zárte and Novella, 2004; Van Opijnen *et al.*, 2007). However, when host heterogeneity arises spatially, then the extent of adaptation to each host cell type strongly depends on the rate of migration among different host (Cuevas *et al.*, 2003). Migration among heterogeneous host types selects for generalist viruses with increased fitness in all the alternative hosts. By contrast, in the absence of migration, viral populations become specialist for their host cell type. This result supports the general view that migration among hosts must be sufficiently low relative to the strength of selection to generate local adaptation to each host (Brown and Pavlovic, 1992; Holt, 1996; Kawecki, 2000). Actually, the conditions for the coexistence of specialist in a heterogeneous environment are very restrictive. If the selective differences among hosts are not so large, as it might be the case for plants belonging to the same family, the balance of production from each host must be roughly equal in order to maintain diversity (Maynard Smith and Hoekstra, 1980; Van Tienderen, 1991). This implies that there must be lots of opportunities for generalists to evolve in heterogeneous environments, even if selection favors specialization to the most productive host in the short term. In good agreement with the above *in vitro* studies in constant environments, it has been recently shown that following serial passages in an herbaceous host (*Pisum sativum*), PPV increased its infectivity, viral load, and virulence in the new host with a concomitant reduction in transmission efficiency in peaches, the original host (Wallis *et al.*, 2007), suggesting that host-range expansion is a costly trait.

Here, it was found that host plant susceptibility was negatively associated with virus' virulence. In other words, virulent viruses were, on average, associated with more resistant plants, whereas less virulent viruses were so with more sensitive plants. This correlation supports the existence of a gene-for gene coevolutionary dynamic between plants and their viruses. Evidences of this sort of interactions between plants and plant-

pathogens have been previously described (Allen *et al.*, 2004). Despite lacking a somatically adaptive immune system, infectious disease resistance in plants is a complex process that provides many potential barriers to virus invasion (resistance genes, hypersensitive local responses, RNA silencing, etc). Because its specificity and adaptability mimic vertebrate's immune system (Voinnet, 2001; Waterhouse *et al.*, 2001; Lecellier and Voinnet, 2004), gene silencing is an especially interesting mechanism. However, until specific mathematical models exploring the interaction between gene silencing and virus infection and virulence will be proposed, models exploring the evolution of virulence under the pressure of immune system may provide some insights. André *et al.* (2003) showed that when a parasite gets engaged in a race with the immune system, the former evolves to provoke more virulent but shorter infections in strongly immunized hosts, a prediction that well matches our observation of more virulent viruses associated with more resistant plants. Indeed, this predicted pattern has also been observed in wild populations of the *Linum marginale* - *Melampsora lini* pathosystem (Thrall and Burdon, 2003). In this example, more virulent *M. lini* rusts were found in association with highly resistant *L. marginale* populations, whereas avirulent pathogens dominated in populations of susceptible plant.

In conclusion, a comparative approach similar to the one here employed can be an useful tool to identify general patterns regarding the infectivity, symptomatology and virulence of plant pathogens and how these factors relate with host properties.

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